



## **ENVIRONMENTAL PROTECTION AGENCY**

**[EPA-HQ-OPPT-2014-0766; FRL-9931-05]**

### **Final Test Guidelines; Endocrine Disruptor Screening Program Test Guidelines**

**(Series 890); Three Tier 2 Non-Mammalian Tests; Notice of Availability**

**AGENCY:** Environmental Protection Agency (EPA).

**ACTION:** Notice.

**SUMMARY:** EPA is announcing the availability of three Office of Chemical Safety and Pollution Prevention (OCSPP) final test guidelines: Medaka Extended One-generation Reproduction Test (MEOGRT), OCSPP Test Guideline 890.2200; Larval Amphibian Growth and Development Assay (LAGDA), OCSPP Test Guideline 890.2300; and Avian Two-generation Toxicity Test in the Japanese Quail (JQTT), OCSPP Test Guideline 890.2100. These test guidelines are part of a series of test guidelines established by OCSPP for use in testing pesticides and chemical substances. The test guidelines serve as a compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions. The test guidelines provide guidance for conducting the test and are also used by EPA, the public, and companies that submit data to EPA.

**FOR FURTHER INFORMATION CONTACT:** Sharlene Matten, telephone number: (202) 564-0130, email address: [matten.sharlene@epa.gov](mailto:matten.sharlene@epa.gov) or Jane Robbins, telephone number: (202) 564-6625; email address: [robbins.jane@epa.gov](mailto:robbins.jane@epa.gov), Office of Science Coordination and Policy (7201M), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001.

### **SUPPLEMENTARY INFORMATION:**

#### **I. Introduction**

EPA is announcing the availability of three final test guidelines: Medaka Extended One-generation Reproduction Test (MEOGRT), OCSPP Test Guideline 890.2200; Larval Amphibian Growth and Development Assay (LAGDA), OCSPP Test Guideline 890.2300; and Avian Two-generation Toxicity Test in the Japanese Quail (JQTT), OCSPP Test Guideline 890.2100. These test guidelines are part of a series of test guidelines established by OCSPP for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Federal Food, Drug, and Cosmetic Act (FFDCA) section 408 (21 U.S.C. 346a), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*), and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601 *et seq.*). The test guidelines serve as a

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compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA.

The test guidelines provide guidance for conducting the test and are also used by EPA, the public, and companies that are subject to data submission requirements under TSCA, FIFRA, and/or FFDCA. As guidance documents, the test guidelines are not binding on either EPA or any outside parties and EPA may depart from the test guidelines where circumstances warrant and without prior notice. At places in this guidance, the Agency uses the word “should.” In this guidance, use of “should” with regard to an action means that the action is recommended rather than mandatory. The procedures contained in the test guidelines are recommended for generating the data that are the subject of the test guideline, but EPA recognizes that departures may be appropriate in specific situations. You may propose alternatives to the recommendations described in the test guidelines and the Agency will assess them for appropriateness on a case-by-case basis.

## **II. General Information**

### *A. Does this Action Apply to Me?*

This action is directed to the public in general. Although this action may be of particular interest to those persons who are or may be required to conduct testing of pesticides and chemical substances for submission to EPA under TSCA, FIFRA, and/or FFDCA, the Agency has not attempted to describe all the specific entities that may be affected by this action.

### *B. How Can I Get Copies of this Document and Other Related Information?*

1. *Docket for this document.* The docket for this action, identified by docket identification (ID) number EPA-HQ-OPPT-2014-0766 is available at <http://www.regulations.gov> or at the Office of Pollution Prevention and Toxics Docket (OPPT Docket), Environmental Protection Agency Docket Center (EPA/DC), EPA West Bldg., Rm. 3334, 1301 Constitution Ave., NW., Washington, DC. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566-1744 and the telephone number for the OPPT Docket is (202) 566-0280. Please review the visitor instructions and additional information about the docket available at <http://www.epa.gov/dockets>.

2. *Electronic access to the OCSPP test guidelines.* To access OCSPP test guidelines electronically, please go to <http://www.epa.gov/ocspp/pubs/frs/home/testmeth.htm>. You may also access the test guidelines in <http://www.regulations.gov>, grouped by series under docket ID numbers: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159 and EPA-HQ-OPPT-2009-0576.

## **III. Overview**

### *A. What Action is EPA Taking?*

EPA is announcing the availability of three final test guidelines that are being added to its 890 Series, entitled “Endocrine Disruptor Screening Program Test Guidelines” and identified as follows:

1. OCSPP Test Guideline 890.2200, entitled *Endocrine Disruptor Screening Program Test Guidelines; Medaka Extended One-generation Reproduction Test*.
2. OCSPP Test Guideline 890.2300, entitled *Endocrine Disruptor Screening Program Test Guidelines; Larval Amphibian Growth and Development Assay*.
3. OCSPP Test Guideline 890.2100, entitled *Endocrine Disruptor Screening Program Test Guidelines; Avian Two-generation Toxicity Test in the Japanese Quail*.

*B. How Were These Final Test Guidelines Developed?*

On January 30, 2015, the Agency released three draft non-mammalian test guidelines for public review and comment as described in a **Federal Register** notice (80 FR 5107) (FRL-9919-43) (Ref. 1). These three draft test guidelines were subsequently revised based on public comments, existing EPA test guidelines, and concurrent Organisation for Economic Co-operation and Development (OECD) test guidelines for MEOGRT (Ref. 2) and LAGDA (Ref. 3).

Comments were submitted by representatives from the following organizations: Endocrine Policy Forum, SynTech Research Laboratory Services, American Petroleum Institute, Wildlife International, People for Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, and the Humane Society of the United States. Comments were grouped according to each test guidelines: JQTT (890.2100), MEOGRT (890.2200) and LAGDA (890.2300).

EPA worked with the OECD to harmonize test guidelines for MEOGRT (Ref. 2) and LAGDA (Ref. 3). The OECD test guidelines were approved at the Working Group of National Coordinators of the Test Guidelines Programme (WNT) and endorsed by the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology in June 2015 prior to EPA finalizing the U.S. MEOGRT and LAGDA test guidelines. EPA revised the terminology, procedures, and statistical practices to harmonize the MEOGRT and LAGDA test guidelines with analogous OECD’s test guidelines and at the same time incorporated public comments received that were not already addressed in the harmonization.

A summary of the substantive changes reflected in the final U.S. MEOGRT and LAGDA test guidelines is provided:

1. The option for extending the MEOGRT through F2 generation reproduction has been removed from the final test guideline pending additional data. The test will end following hatching of the F2 offspring. This is consistent with the decision made in the draft OECD test guideline for MEOGRT. This test guideline may be updated as new information and data are considered. For example, guidance on extending the F2 generation through reproduction may

be potentially useful under certain circumstances (*e.g.*, chemicals with high bioconcentration potential or indications of trans-generational effects in other taxa).

2. The mean water temperature over the duration of the MEOGRT has been changed to  $25 \pm 2$  °C to be consistent with the analogous OECD test guideline.

3. The LAGDA developmental stage terminology has been clarified to avoid confusion with what is meant by complete metamorphosis.

4. Clarified the conduct of liver and kidney histopathology in the MEOGRT and LAGDA test guidelines for overt toxicity. An effort was made to clarify and provide more explicit guidance as to what specific histopathology is appropriate based on the results of the study.

5. The rationale for use of solvent control only, dilution water control only, or pooled controls in the statistical analyses for the MEOGRT and LAGDA was clarified.

6. The guidelines have been modified to address commenters' concerns that they be more flexible and less prescriptive. Examples have been provided as appropriate to add clarity.

The JQTT draft test guideline (OCSPP 890.2100) was revised to address comments provided by the public, the draft OECD test guideline for the avian two-generation toxicity test in the Japanese quail (Ref. 4), as well as the existing EPA and OECD test guidelines for avian one-generation toxicity tests (Refs. 5 and 6). EPA revised the terminology, procedures, endpoints measured, figures, tables, and appendices in the JQTT test guideline to clarify specific points raised by public commenters as follows:

1. The revised test guidelines include fewer endpoints. For example, the revisions eliminated behavioral endpoints to reduce the overall numbers of birds required for the study; eliminated endpoints that are difficult to obtain (*i.e.*, hormone levels measured in embryo blood samples); eliminated redundant endpoints; and statistical analyses.

2. For clarity, the test termination point is following measurement of the 14-day survival of filial 2 (F2) generation chicks. This is the minimum length of the study necessary to evaluate and measure a chemical's effect on the F1 generation's reproductive performance. If delayed reproduction is observed in F1 birds, a decision to extend the F2 generation may be made. If extended, the test should be terminated when F2 birds are approximately 6 weeks old when 90% of control animals have reached sexual maturity. The decision to limit the length of the JQTT is consistent with EPA's efforts to move to extended one-generation reproduction test protocols for Tier 2 tests rather than multigenerational studies (Ref. 7). Extended one-generation reproduction tests are technically sound, save animals, and reduce costs.

3. The guidelines have been modified to address commenters' concerns that they be more flexible and less prescriptive. Examples have been provided as appropriate to add clarity.

#### **IV. References**

The following is a listing of the documents that are specifically referenced in this document. The docket includes these documents and other information considered by EPA, including documents that are referenced within the documents that are in the docket, even if the referenced document is not physically located in the docket. For assistance in locating these other documents, please consult the persons listed under **FOR FURTHER INFORMATION CONTACT**.

1. EPA Draft Test Guidelines; Endocrine Disruptor Screening Program Test Guidelines (Series 890); Three Tier 2 Non-Mammalian Tests; Notice of Availability and Public Comment, **Federal Register** (80 FR 5107, January 30, 2015). It is available at <http://www.gpo.gov/fdsys/pkg/FR-2015-01-30/pdf/2015-01836.pdf>.

2. OECD, Test No. 240: Medaka Extended One-Generation Reproduction Test, OECD Guidelines for the Testing of Chemicals, section 2 (2015). It is available at [http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems\\_20745761](http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761).

3. OECD, Test No. 241: Larval Amphibian Growth and Development Test, OECD Guidelines for the Testing of Chemicals, section 2 (2015). It is available at [http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems\\_20745761](http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761).

4. OECD Guidelines for Testing of Chemicals. Proposal for a new test guideline: Avian Two-generation Toxicity Test in the Japanese Quail, Draft November 2005. It is available at [http://www.epa.gov/endo/pubs/edmvac/2gen\\_guide\\_gd\\_draft1.pdf](http://www.epa.gov/endo/pubs/edmvac/2gen_guide_gd_draft1.pdf).

5. U.S. EPA Ecological Effects Test Guidelines; OCSPP 850.2300, Avian Reproduction Test. January 2012. EPA 712C-023. It is available at [http://www.epa.gov/ocspp/pubs/frs/publications/Test\\_Guidelines/series850.htm](http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series850.htm).

6. OECD, Test No. 206: Avian Reproduction Test. OECD Guidelines for the Testing of Chemicals, section 2 – Effect on Biotic Systems (adopted 1984). OECD Publishing, Paris (1993) DOI: It is available at <http://dx.doi.org/10.1787/9789264070028-en>.

7. OECD, Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, section 4, 28 July 2011, DOI. It is available at <http://dx.doi.org/10.1787/9789264122550-en>.

**Authority:** 7 U.S.C. 136 *et seq.*; 15 U.S.C. 2601 *et seq.*; 21 U.S.C. 301 *et seq.*

Dated: August 18, 2015.

Louise P. Wise,

*Assistant Administrator, Office of Chemical Safety and Pollution Prevention.*